1 This investigation received financial support from the UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases, the Fundación Julio Cherny, and the Ministerio de Salud Pública y Medio Ambiente de la República Argentina. The authors gratefully acknowledge the technical assistance of Mr Esteban Bontempi.
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Cone mosaic in a teleost retina: No difference between light and dark adapted states[1]

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Summary. The mosaic arrangement of retinal cones in the eye of the African cichlid fish, *Haplochromis burtoni*, is the same in both light and dark adapted states. This is in contrast to Kunz' claim[2] that the retinal mosaic pattern changes from a square to a row type during dark adaptation, in the guppy *(Poecelia reticulata)*. Kunz' histological procedure may account for this difference in results.

Cone photoreceptors in many teleost retinae are arranged in a highly regular mosaic pattern[3-6]. 2 basic types of mosaic patterns have been described; 'square' and 'row' types. In both patterns, a row of double or twin cones alternates with a row of single cones. The patterns differ in that in one case the double (twin) cones are arranged so that lines through the centers of each pair are at right angles with respect to one another ('square' pattern) whereas in the other case the axes of the double cones are parallel to one another ('row' pattern) (fig. 1, a). The 'square' mosaic (fig. 1, b) is thought to be associated with fish which are highly dependent on vision[3]. In some species, both patterns exist in different regions of adult retina[7,8] or occur at different times during ontogeny[9-11]. Rods are interspersed in the cone arrays throughout the extent of the retina[12].

In the African cichlid fish, *Haplochromis burtoni*, a highly ordered square type mosaic is present[13]. The chromatic organization of these cones, as determined by vital staining with nitro-blue tetrazolium di-formazan, is an alternating symmetry which serves to maximize the chromatic resolution available to the animal[12]. This is consistent with behavioral[14,15], developmental[16], and neuroanatomical[17] evidence that vision is the primary sensory input for *H. burtoni*.

In a recent report, Kunz[2] suggested that the cone mosaic, as reflected in outer segment organization in the guppy (*Poecilia reticulata* P.) changes from a square to a row type during dark adaptation. She removed adult fish retinae by microdissection in Ringer's solution with or without calcium and examined the tissue using light or electron microscopy. At the level of the outer segments, her micrographs show an apparent change in mosaic type from double cone zig-zag rows (fig. 1, b) to parallel rows (fig. 1, a). She suggests that this change supports the hypothesis that zig-zag rows of double cones are more suitable for high acuity vision and parallel rows more suitable for low-level light detection. If this hypothesis were true, such a change in mosaic type should occur in other teleost species dependent on vision during dark adaptation. For this reason, we examined the cone mosaic in the retina of *H. burtoni* under conditions of light and dark adaptation.

Materials and methods. Adult male *Haplochromis burtoni* (7-8 cm long) bred from wild-caught fish[14] were used for the experiments. The animals were maintained at 12:12 light-dark cycle characteristic of their natural habitat in central Africa[14]. Animals were anesthetized by immersion in tricaine methanesulphonate (MS222, Sandoz) at 03.00 h and one eye removed, and the procedure repeated at 15.00 h and the other eye removed. This allowed us to compare 2 eyes from the same animal, differing only in the time of day when they were prepared for histology. Enucleation during the dark portion of the light-dark cycle was performed using an infrared viewing device (FJW, Mt. Prospect, Ill.) so that there was no significant illumination below 750 nm, which is well above the photopigment sensitivity limit[13]. Following enucleation, the lenses were removed and the eyecups were immediately immersed in fixative (1% paraformaldehyde, 2.5% gluteraldehyde, 3% sucrose in 0.06 M PO_4 buffer) with dorsal margin of the eye

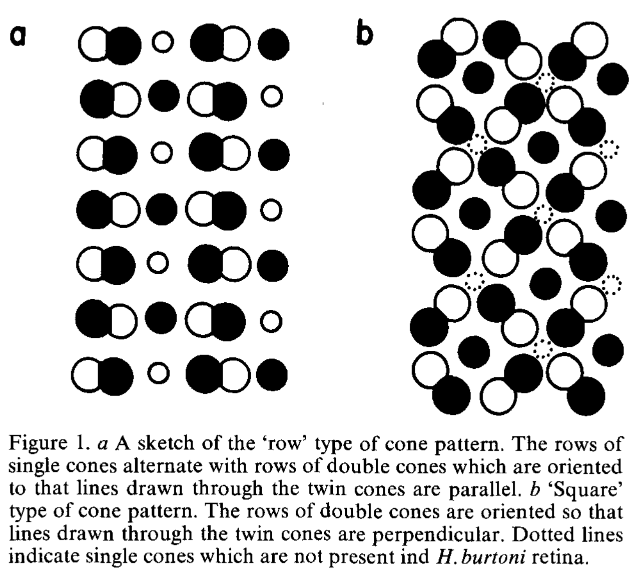

Figure 1. *a* A sketch of the 'row' type of cone pattern. The rows of single cones alternate with rows of double cones which are oriented to that lines drawn through the twin cones are parallel. *b* 'Square' type of cone pattern. The rows of double cones are oriented so that lines drawn through the twin cones are perpendicular. Dotted lines indicate single cones which are not present ind *H. burtoni* retina.

Experientia 38 (1982), Birkhäuser Verlag, CH-4010 Basel/Switzerland

marked to allow orientation during sectioning. The eyecups were post-fixed (1% osmium, 3% sucrose in 0.06 M PO_4 buffer at pH 7.3), washed, and stored overnight in 0.2 M cacodylate buffer, pH 7.3. Following washing and dehydration, the eyecups were embedded in epon. Sections were stained with 0.05% toluidine blue and sectioned at 3 μm intervals.

To examine the retinal mosaic, tangential sections through the margin of the eye were used, and to confirm the state of retinomotor movement, sections near the midline were examined.

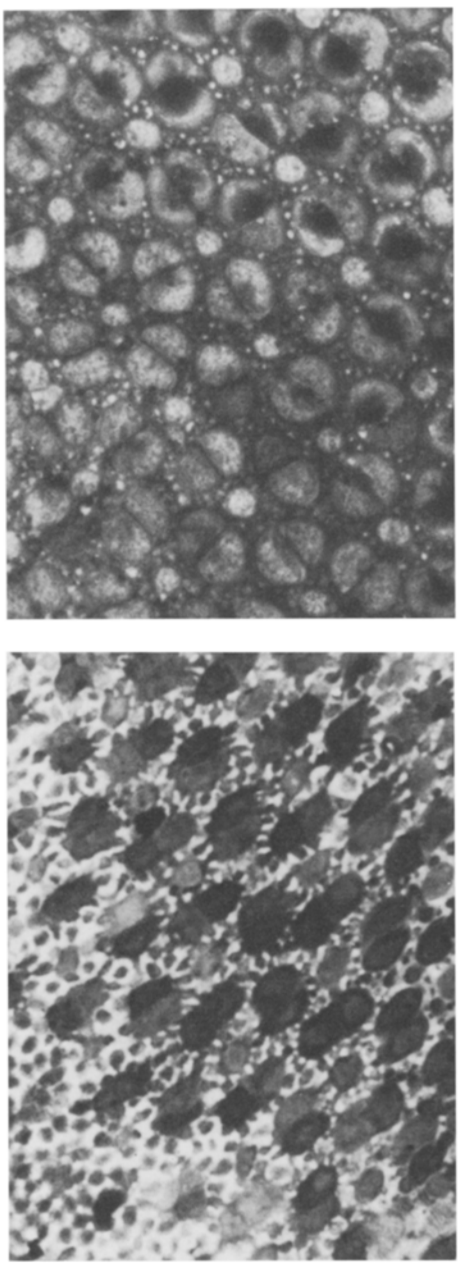

Figure 2. *a* Cone photoreceptor mosaic in the African cichlid fish, *Haplochromis burtoni*. Tangential section at the level of the outer segment. This retinal tissue was prepared from an eye removed while it was light-adapted (see text). *b* Cone photoreceptor mosaic prepared during dark adaptation. This eye is from the fish as in figure 2,a, removed 12 h earlier (see text for details).

Results and discussion. Viewing the retinal sections under a light microscope revealed that the square mosaic arrangement of retinal cones is present in both day and night adapted states of the retinae (fig. 2, a and b). In light-adapted retinae (fig. 2,a), the square mosaic pattern is easier to visualize because the outer segment of the single cone can be seen in the same plane of section as the outer segments of the twin cone members. In the dark-adapted retina (fig. 2,b), the square pattern is also evident. The square matrix found in the dark-adapted animal is slightly skewed but nonetheless still is distinctly of the type shown schematically in figure 1,b.

In summary, there is no difference in the mosaic pattern between retinae which are light-adapted and those which are dark-adapted. What could account for the differences between these results and those of Kunz? First, it could be that this difference reflects a true species difference between the African cichlid fish, *Haplochromis burtoni*, and the guppy, *Poecilia reticulata*. This seems unlikely, given the high levels of similarity in numerous other aspects of teleost fish retinae[5]. Although this possibility cannot be excluded by the present evidence, a second, more parsimonious explanation seems appropriate. The preparation of the tissue can greatly affect the stability and apparent order within retinal tissue. In addition, the plane of section can alter the appearance of the mosaic. For comparison, we prepared retinae as above, using Bouin's fixative rather than the paraformaldehyde-gluteraldehyde combination. Although the results are the same as those described here, the amount of 'wobble' in the matrix pattern is significantly larger. This is reasonable because Bouin's fixative penetrates more slowly. The cone outer segments are fragile and may easily be disrupted by the procedures used for their examination. It seems most likely that the use of calcium-free Ringer's solution to expedite dissection, and whole retina dissection, contribute to the apparent appearance of row-type matrices in a guppy retina in Kunz' work. By fixing the tissue immediately following sacrifice, embedding and sectioning, it seems reasonable that the results we obtained here could be replicated in the guppy. We have attempted to obtain whole-mounted retinae in the fashion described[2] and found an obvious increase in the irregularity of the cone mosaic pattern. This may easily be attributed to the tissue preparation and not to an intrinsic property of the retina itself.

Teleost fish probably have mosaic retinae because the dioptric power of the eye is vested entirely in the spherical fish lens, which favors the retina being close to that lens[12]. To achieve adequate visual acuity in such a retina, the photoreceptor elements must be closely spaced and presumably the packing optimized. As the eyes enlarge, the spacing between cones can increase without loss of acuity, because the larger lens produces a larger image. The 'square' matrix is better suited for visual discrimination, assuming the members of the twin can function independently, since their orthogonal relationship increases the inherent range of sensitivity of the photoreceptors. That is, a line moving across the retina which is parallel to the long axis of one pair of cones will intersect the next pair at right angles to its direction of movement, making the line easier to localize.

It seems unlikely that teleost fishes would change their mosaic type through rotation of the cone outer segments in the dark. The outer segments are surrounded by pigment epithelium and almost certainly do not collect much light. The apparent change in mosaic type during dark adaptation may more parsimoniously be ascribed to histological preparation which relieves the need for postulating intricate reasons or mechanisms to achieve rotation of the twin cones during retinomotor activity.